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Enclosed are certified copies of four priority documents for the above named application. Applicants hereby claim priority under 35 U.S.C. §§119 from the following applications:

GB9604709.7 filed March 5, 1996;

GB9605725.2 filed March 19, 1996;

GB9603325.3 filed February 16, 1996; and,

GB9625334.9 filed December 5, 1996.

Acknowledgment of the claim of priority and receipt of the certified copies is requested.

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Les documents ci-annexés sont conformes aux documents figurant dans le dossier de la demande de brevet dont le numéro est indiqué ci-dessous (règle 94(4) CBE).

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Patents ADP number *(if you know it)* 06614690021

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4. Title of the invention — A COMPOUND

5. Name of your agent *(if you have one)* — D YOUNG & CO

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A COMPOUND

The present invention relates to a compound.

In particular the present invention relates to a pharmaceutical composition comprising the compound.

Breast and endometrial cancers are major causes of death in Western women. In particular, tumours in endocrine-dependent tissues, such as the breast and endometrium, occur most frequently in postmenopausal women at a time when the ovaries have ceased their production of oestrogens.

Evidence suggests that oestrogens are the major mitogens involved in stimulating and promoting the growth of tumours in endocrine-dependent tissues, such as the breast and endometrium[21]. Although plasma oestrogen concentrations are similar in women with or without breast cancer, breast tumour oestrone and oestradiol levels are significantly higher than in normal breast tissue or blood. In addition, in postmenopausal women oestrogens continue to be produced by extraglandular production in adipose tissue but also in normal and malignant breast tissues[22].

Figures 1 and 2 are schematic diagrams showing some of the enzymes involved in the *in situ* synthesis of oestrone from oestrone sulphate, oestradiol and androstenedione.

In Figure 2, which schematically shows the origin of oestrogenic steroids in postmenopausal women, "ER" denotes Oestrogen Receptor, "DHA/-S" denotes Dehydroepiandrosterone/-Sulphate, "Adiol" denotes Androstenediol, "E1-STS" denotes Oestrone Sulphatase, "DHA-STS" denotes DHA-sulphatase, "Adiol-STS" denotes Adiol Sulphatase, and "17B-HSD" denotes Oestradiol 17B-hydroxysteroid dehydrogenase.

As can be seen, the main two enzymes that are involved in the peripheral synthesis of oestrogens are the aromatase enzyme and the enzyme oestrone sulphatase.

In short, the aromatase enzyme converts androstenedione, which is secreted in large amounts by the adrenal cortex, to oestrone. Recent reports have suggested that some flavones could inhibit aromatase activity[35,36].

Much of the oestrone so formed, however, is converted to oestrone sulphate (E1S) and there is now a considerable body of evidence showing that E1S in plasma and tissue acts as a reservoir for the formation of oestrone by the action of oestrone sulphatase[23].

In this regard, it is now believed that the oestrone sulphatase (E1-STS) pathway - i.e. the hydrolysis of oestrone sulphate to oestrone (E1S to E1) is the major source of oestrogen in breast tumours[1,2]. This theory is supported by a modest reduction of plasma oestrogen concentration in postmenopausal women with breast cancer treated by aromatase inhibitors, such as aminoglutethimide and 4-hydroxyandrostenedione[3,4] and also by the fact that plasma E1S concentration in these aromatase inhibitor-treated patients remains relatively high. The long half-life of E1S in blood (10-12 h) compared with the unconjugated oestrogens (20 min)[5] and high levels of steroid sulphatase activity in liver and, normal and malignant breast tissues, also lend support to this theory[6].

Thus, oestrogen formation in malignant breast and endometrial tissues *via* the sulphatase pathway makes a major contribution to the high concentration of oestrogens which are present in these tumours[24,25].

PCT/GB92/01587 teaches novel steroid sulphatase inhibitors and pharmaceutical compositions containing them for use in the treatment of oestrone dependent tumours, especially breast cancer. These steroid sulphatase inhibitors are sulphamate esters, such as N,N-dimethyl oestrone-3-sulphamate and, preferably, oestrone-3-sulphamate (otherwise known as "EMATE").

EMATE is a potent E1-STS inhibitor as it displays more than 99% inhibition of E1-STS activity in intact MCF-7 cells at 0.1 μM. EMATE also inhibits the E1-STS enzyme in a time-dependent and concentration-dependent manner, thereby indicating that it acts as an active site-directed inactivator[7,8].

Although EMATE was originally designed for the inhibition of E1-STS, it also inhibits dehydroepiandrosterone sulphatase (DHA-STS), which is an enzyme that is believed to have a pivotal role in regulating the biosynthesis of the oestrogenic steroid androstenediol[8,9]. This is of significance as there is now evidence to suggest that androstenediol may be of even greater importance as a promoter of breast tumour growth[10].

EMATE is also active *in vivo* as almost complete inhibition of rat liver E1-STS (99%) and DHA-STS (99%) activities resulted when it is administered either orally or subcutaneously[11]. In addition, EMATE has been shown to have a memory enhancing effect in rats[14]. Studies in mice have suggested an association between DHA-STS activity and the regulation of part of the immune response. It is thought that this may also occur in humans[15,16]. The bridging *O*-atom of the sulphamate moiety in EMATE is believed to be important for inhibitory activity. Thus, when the 3-*O*-atom is replaced by other heteroatoms - as in oestrone-3-*N*-sulphamate and oestrone-3-*S*-sulphamate - these analogues are weaker non-time-dependent inactivators[12].

Thus, EMATE is a potent steroid sulphatase inhibitor which blocks the hydrolysis of both E1S and DHA-S[29-31]. This inhibitor, therefore, not only blocks the synthesis of oestrone from E1S but also the formation of androstenediol from DHA-S.

In addition to oestrone, the other major steroid with oestrogenic properties which is produced by postmenopausal women is androstenediol (see Figure 2).

Androstenediol, although an androgen, can bind to the oestrogen receptor (ER) and can stimulate the growth of ER positive breast cancer cells and the growth of carcinogen-induced mammary tumours in the rat[26,27]. Importantly, in postmenopausal women 90% of the androstenediol produced originates from the androgen dehydroepiandrosterone sulphate (DHA-S) which is secreted in large amounts by the adrenal cortex. DHA-S is converted to DHA by DHA sulphatase, which may be the same as, or different from, the enzyme, oestrone sulphatase, which is responsible for the hydrolysis of E1S[28].

During the last 10-15 years considerable research has also been carried out to develop potent aromatase inhibitors, some of which are currently undergoing clinical evaluation. However, in three recent reports of postmenopausal women with breast cancer who received aromatase inhibitor therapy, plasma E1S concentrations remained between 400-1000 pg/ml[32-34].

In summation therefore *in situ* synthesis of oestrogen is thought to make an important contribution to the high levels of oestrogens in tumours and therefore specific inhibitors of oestrogen biosynthesis are of potential value for the treatment of endocrine-dependent tumours.

Moreover, even though oestrogen formation in malignant breast and endometrial tissues *via* the sulphatase pathway makes a major contribution to the high concentration of oestrogens, there are still other enzymatic pathways that contribute to *in vivo* synthesis of oestrogen.

Thus, there is an urgent need to develop new therapies for the treatment of these cancers.

The present invention therefore seeks to overcome one or more of the problems associated with the prior art methods of treating breast and endometrial cancers.

According to a first aspect of the present invention there is provided a sulphamate compound suitable for use as an inhibitor of both oestrone sulphatase activity and aromatase activity.

In a highly preferred embodiment, the compound of the present invention is a non-steroidal compound.

According to a second aspect of the present invention there is provided a compound having the general formula II wherein F represents a phenolic ring structure (a first ring structure), J represents a third ring structure, I represents a phenolic ring structure (a second ring structure), G is an optional double bond, H is a link joining the second ring structure to the third ring structure, and Y represents a suitable second group; wherein any one of ring structures F, J and I has bound thereto a sulphamate group.

According to a third aspect of the present invention there is provided a compound according to the present invention for use as a pharmaceutical.

According to a fourth aspect of the present invention there is provided a compound according to the present invention for inhibiting oestrone sulphatase activity and aromatase activity.

According to a fifth aspect of the present invention there is provided a pharmaceutical composition comprising a compound according to the present invention; and a pharmaceutically acceptable carrier, excipient or diluent.

According to a sixth aspect of the present invention there is provided the use of a compound according to the present invention in the manufacture of a pharmaceutical for inhibiting oestrone sulphatase activity and aromatase activity.

According to a seventh aspect of the present invention there is provided a process for preparing a compound according to the present invention, the process comprising sulphating a flavone, isoflavone or a flavanone.

According to an eighth aspect of the present invention there is provided a process for preparing a compound according to the present invention, the process comprising sulphamaylating a flavone, isoflavone or a flavanone.

In one aspect, therefore, the present invention provides a compound, or a pharmaceutical composition comprising the same, that can affect, such as substantially inhibit, not only the oestrone sulphatase pathway - which pathway converts oestrone to and from oestradiol - but also the aromatase pathway - which pathway converts the androgen precursor androstenedione to oestrone.

This aspect of the present invention is advantageous because by the administration of one type of compound it is possible to block the synthesis of oestrone from both androstenedione and E1S.

In addition, the present invention is further advantageous in that it may also be possible to block the formation of androstenediol from DHA-S.

Hence, the present invention provides compounds that have considerable therapeutic advantages, particularly for treating breast and endometrial cancers.

The compounds of the present invention are different from those disclosed in the prior art because they can act as therapeutic agents that possess both aromatase and steroid sulphatase inhibitory properties.

In a preferred embodiment the compound of the present invention comprises a first ring structure and a sulphamoyl group, which first ring structure may be substituted and/or unsaturated.

Preferably the first ring structure is a phenolic ring structure, which phenolic ring may be substituted.

Preferably, the compound of the present invention further comprises a second ring structure, which second ring structure may be substituted and/or unsaturated.

Preferably the second ring structure is a phenolic ring structure, which phenolic ring may be substituted.

Preferably, the compound of the present invention further comprises a third ring structure which is intermediate the first ring structure and the second ring structure, which third ring structure may be substituted and/or unsaturated.

The present invention will now be described by reference to the Formulae presented in Figures 3-9.

In this regard, its is generally preferred that the compound of the present invention has the general formula I wherein A represents the first ring structure, B represents the third ring structure, D represents the second ring structure, C is an optional double bond, E is a link joining the second ring structure to the third ring structure, X represents a suitable first group, and Y represents a suitable second group; wherein any one of ring structures A, B and D is a phenolic ring; and wherein any one of ring structures A, B and D has bound thereto a sulphamate group.

Each of the ring structures can independently comprise from 3 to 20 atoms in the ring, preferably from 4 to 8 atoms in the ring. Preferably, ring A and ring D comprise 6 atoms in the ring.

Preferably, the first ring structure and the second ring structure are substituted.

Preferably, any one of ring structures A and D has bound thereto a sulphamate group.

Preferably, each of the first ring and the second ring is a homogeneous ring structure - i.e. the ring is made up of the same atoms.

Preferably, each of the first ring and the second ring comprises only carbon atoms in the ring.

Preferably, X is C=O.

Preferably, the compound of the present invention has the general formula II wherein F represents a phenolic ring structure (the first ring structure), J represents the third ring structure, I represents a phenolic ring structure (the second ring structure), G is an optional double bond, H is a link joining the second ring structure to the third ring structure, and Y represents a suitable second group; wherein any one of ring structures F, J and I has bound thereto a sulphamate group.

Preferably, the third ring structure is a heterogeneous ring structure - i.e. different atoms are in the ring.

Preferably, Y is O.

Preferably any one of the ring structures F and I has bound thereto a sulphamate group.

Preferably, link E or link H is a bond.

Preferably, the compound of the present invention is a sulphamate of any one of a flavone, an isoflavone or a flavanone.

Preferably, the compound of the present invention is any one of a compound of the general formula IV, a compound of the general formula V, or a compound of the general formula VI; wherein R_1-R_{12} are independently selected from H, OH, a halogen, an amine, an amide, a sulphonamine, a sulphonamide, any other sulphur containing group, a saturated or unsaturated C_{1-10} alkyl, an aryl group, a saturated or unsaturated C_{1-10} ether, a saturated or unsaturated C_{1-10} ester, a phosphorous containing group; and wherein at least one of R_1-R_{12} is a sulphamate group.

Preferably, the sulphamate group has the general formula $OSO_2NR_{13}R_{14}$ wherein R_{13} and R_{14} are independently selected from H, OH, a halogen, a saturated or unsaturated C_{1-10} alkyl, an aryl group, a saturated or unsaturated C_{1-10} ether, a saturated or unsaturated C_{1-10} ester.

Preferably, the compound of the present invention is any one of a compound of the general formula IV, a compound of the general formula V, or a compound of the general formula VI; wherein R_1-R_{12} are independently selected from H, OH, $OSO_2NR_{13}R_{14}$, O-CH_3; wherein at least one of R_1-R_{12} is $OSO_2NR_{13}R_{14}$, and wherein R_{13} and R_{14} are defined as above.

Preferably, each of R_{13} and R_{14} is H.

Preferably, the compound of the present invention is a sulphamate of any one of the flavone of formula VII, the isoflavone of formula VIII, or the flavanone of formula IX.

Preferably, the compound of the present invention is the sulphamate of any one of formula VII, formula VIII or formula IX.

Preferably, the compound of the present invention is a sulphamate of any one of a flavone, an isoflavone or a flavanone; and wherein the sulphamoyl group is on the C4' atom of the flavone, isoflavone or flavanone. The C4' position has been shown in general Formula III according to the present invention.

Preferably, the compound of the present invention is a flavonoid or flavanoid sulphamate.

In summation the present invention provides compounds that avoid the need for polytherapy. In this regard, the compounds of the present invention can act as therapeutic agents that possess both aromatase and steroid sulphatase inhibitory properties.

Preferably, the compound is hydrolysable by an enzyme having steroid sulphatase (E.C. 3.1.6.2) activity.

The present invention will now be described only by way of example.

COMPOUNDS SYNTHESISED

The following sulphamate derivatives were synthesised from the following parent compounds:

PARENT COMPOUND	SULPHAMATE COMPOUND
1	2
3	4
5	6
7	8
9	10

wherein

1 = 6-hydroxy flavone

2 = flavone-6-sulphamate

3 = 7-hydroxy flavone

4 = flavone-7-sulphamate

5 = 5,7-dihydroxy flavone

6 = 5-hydroxy-flavone-7-sulphamate

7 = 5,7-dihydroxy-4'-hydroxy-flavone

8 = 5,7-dihydroxy flavanone-4'-flavanone sulphamate

9 = 5,7-dihydroxy-4'-methoxy-isoflavone

10 = 5-hydroxy-4'-methoxy-isoflavone-isoflavone-7-sulphamate

The formulae are presented in Figures 7-9.

SYNTHESIS

The sulphamate derivatives were prepared essentially as described previously[29]. In this regard, solutions of the appropriate flavone, isoflavone or flavanone were treated in anhydrous DMF with sodium hydride (1 equiv) at 0°C (under N_2). Sulphamoyl chloride (ca 1.5 equiv) was then added, and after the mixture was warmed to room temperature overnight and the reaction quenched, the crude product, after work up for each flavone, isoflavone or flavanone, was purified by flash chromatography and recrystallization. All compounds were fully characterised by spectroscopic and combustion analysis.

ASSAY OF INHIBITION OF SULPHATASE AND AROMATASE ACTIVITIES

Sulphatase inhibition was assessed using placental microsome (100,000 g) preparations or intact MCF-7 breast cancer cells as described previously[29,30]. Placental microsomes were incubated with ^{3}H E1S, adjusted to 20 μM with unlabelled substrate, in the absence or presence of inhibitor.

Placental microsomes were also used to assess the aromatase inhibitory properties of the flavanoid sulphamates using a tritiated water release assay[37]. Further placental microsomes (200 μl) were incubated with [1β-^{3}H] androstenedione, 60 nM and 1 mM NADPH in the absence or presence of inhibitor.

INHIBITION OF SULPHATASE AND AROMATASE ACTIVITIES

Inhibition of oestrone sulphatase and aromatase activities in placental microsomes by the flavanoid sulphamate derivatives is shown in the Table below.

	CONCENTRATION	% INHIBITION	% INHIBITION
COMPOUND			
	μM	Sulphatase	Aromatase
Flavone-6-sulphamate	1 10	26.8 89.5	1 6.5
Flavone-7-sulphamate	1 10 50 100	- - 56.3 75.3	55 86
5-hydroxy flavone-7-sulphamate	1 10	8 21	5 76
5,7-dihydroxy flavanone 4'-sulphamate	0.1 1 10	30.4 79.1 98.1	Not tested Not tested Not tested
5-hydroxy-4'-methoxy-isoflavone-7-sulphamate	1 10	1 50.6	2 5

From the results, it can be seen that potent inhibition of sulphatase and aromatase activities was detected. For sulphatase inhibition this ranged from 21% at 10 μM by 5-hydroxy flavone-7-sulphamate, to 98% by 5,7-dehydroxy flavanone-4'-sulphamate at 10 μM. Potent aromatase inhibition was also achieved ranging from 6.5% by flavone-6-sulphamate at 10 μM to 86% by flavone-7-sulphamate at 10 μM.

Other modifications of the present invention will be apparent to those skilled in the art.

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CLAIMS

1. A sulphamate compound suitable for use as an inhibitor of both oestrone sulphatase activity and aromatase activity.

2. A compound according to claim 1 wherein the compound comprises a first ring structure and a sulphamoyl group, which first ring structure may be substituted and/or unsaturated.

3. A compound according to claim 2 wherein the first ring structure is a phenolic ring structure, which phenolic ring may be substituted.

4. A compound according to any one of claims 1 to 3 wherein the compound further comprises a second ring structure, which second ring structure may be substituted and/or unsaturated.

5. A compound according to claim 4 wherein the second ring structure is a phenolic ring structure, which phenolic ring may be substituted.

6. A compound according to any one of claims 1 to 5 wherein the compound further comprises a third ring structure which is intermediate the first ring structure and the second ring structure, which third ring structure may be substituted and/or unsaturated.

7. A compound according to claim 6 wherein the compound has the general formula I; wherein A represents the first ring structure, B represents the third ring structure, D represents the second ring structure, C is an optional double bond, E is a link joining the second ring structure to the third ring structure, X represents a suitable first group, and Y represents a suitable second group; wherein any one of ring

structures A, B and D is a phenolic ring; and wherein any one of ring structures A, B and D has bound thereto a sulphamate group.

8. A compound according to claim 7 wherein the compound has the general formula II wherein F represents a phenolic ring structure (the first ring structure), J represents the third ring structure, I represents a phenolic ring structure (the second ring structure), G is an optional double bond, H is a link joining the second ring structure to the third ring structure, and Y represents a suitable second group; wherein any one of ring structures F, J and I has bound thereto a sulphamate group.

9. A compound having the general formula II wherein F represents a phenolic ring structure (a first ring structure), J represents a third ring structure, I represents a phenolic ring structure (a second ring structure), G is an optional double bond, H is a link joining the second ring structure to the third ring structure, and Y represents a suitable second group; wherein any one of ring structures F, J and I has bound thereto a sulphamate group.

10. A compound according to any one of claims 7 to 9 wherein the first ring structure and the second ring structure are substituted.

11. A compound according to any one of claims 7 to 10 wherein any one of ring structures A or F and D or I has bound thereto a sulphamate group.

12. A compound according to any one of claims 7 to 11 wherein the third ring structure is a heterogeneous ring structure.

13. A compound according to any one of claims 7 to 12 wherein X is C=O.

14. A compound according to any one of claims 7 to 13 wherein Y is O.

15. A compound according to any one of the preceding claims wherein the compound of the present invention is a sulphamate of any one of a flavone, an isoflavone or a flavanone.

16. A compound according to claim 15 wherein the compound is any one of: a compound of the general formula IV, a compound of the general formula V or a compound of the general formula VI; wherein R_1-R_{12} are independently selected from H, OH, a halogen, an amine, an amide, a sulphonamine, a sulphonamide, any other sulphur containing group, a saturated or unsaturated C_{1-10} alkyl, an aryl group, a saturated or unsaturated C_{1-10} ether, a saturated or unsaturated C_{1-10} ester, a phosphorous containing group; and wherein at least one of R_1-R_{12} is a sulphamate group.

17. A compound according to any one of the preceding claims wherein the sulphamate group has the general formula $OSO_2NR_{13}R_{14}$ wherein R_{13} and R_{14} are independently selected from H, OH, a halogen, a saturated or unsaturated C_{1-10} alkyl, an aryl group, a saturated or unsaturated C_{1-10} ether, a saturated or unsaturated C_{1-10} ester

18. A compound according to any one of the preceding claims wherein the compound is any one of a compound of the general formula IV, a compound of the general formula V or a compound of the general formula VI; wherein R_1-R_{12} are independently selected from H, OH, $OSO_2NR_{13}R_{14}$, O-CH_3; wherein at least one of R_1-R_{12} is $OSO_2NR_{13}R_{14}$, and wherein R_{13} and R_{14} are defined in claim 17.

19. A compound according to claim 16 or claim 17 wherein R_{13} and R_{14} are H.

20. A compound according to any one of the preceding claims wherein the compound is a sulphamate of any one of the flavone of formula VII, the isoflavone of formula VIII or the flavanone of formula IX.

21. A compound according to any one of the preceding claims wherein the compound is the sulphamate of any one of formula VII, formula VIII or formula IX.

22. A compound according to any one of the preceding claims wherein the compound is a sulphamate of any one of a flavone, an isoflavone or a flavanone; and wherein the sulphamoyl group is on the C4' atom of the flavone, isoflavone or flavanone.

23. A compound according to any one of the preceding claims wherein the compound is a flavanoid sulphamate.

24. A compound according to any one of the preceding claims for use as a pharmaceutical.

25. A compound according to any one of claims 1 to 23 for inhibiting oestrone sulphatase activity and aromatase activity.

26. A pharmaceutical composition comprising a compound according to any one of claims 1 to 23; and a pharmaceutically acceptable carrier, excipient or diluent.

27. Use of a compound according to any one of claims 1 to 23 in the manufacture of a pharmaceutical for inhibiting oestrone sulphatase activity and aromatase activity.

28. A process for preparing a compound according to any one of claims 1 to 23, the process comprising sulphating a flavone, isoflavone or a flavanone.

29. A process for preparing a compound according to any one of claims 1 to 23, the process comprising sulphamaylating a flavone, isoflavone or a flavanone.

30. A compound substantially as described herein.

31. A process of preparing a compound substantially as described herein.

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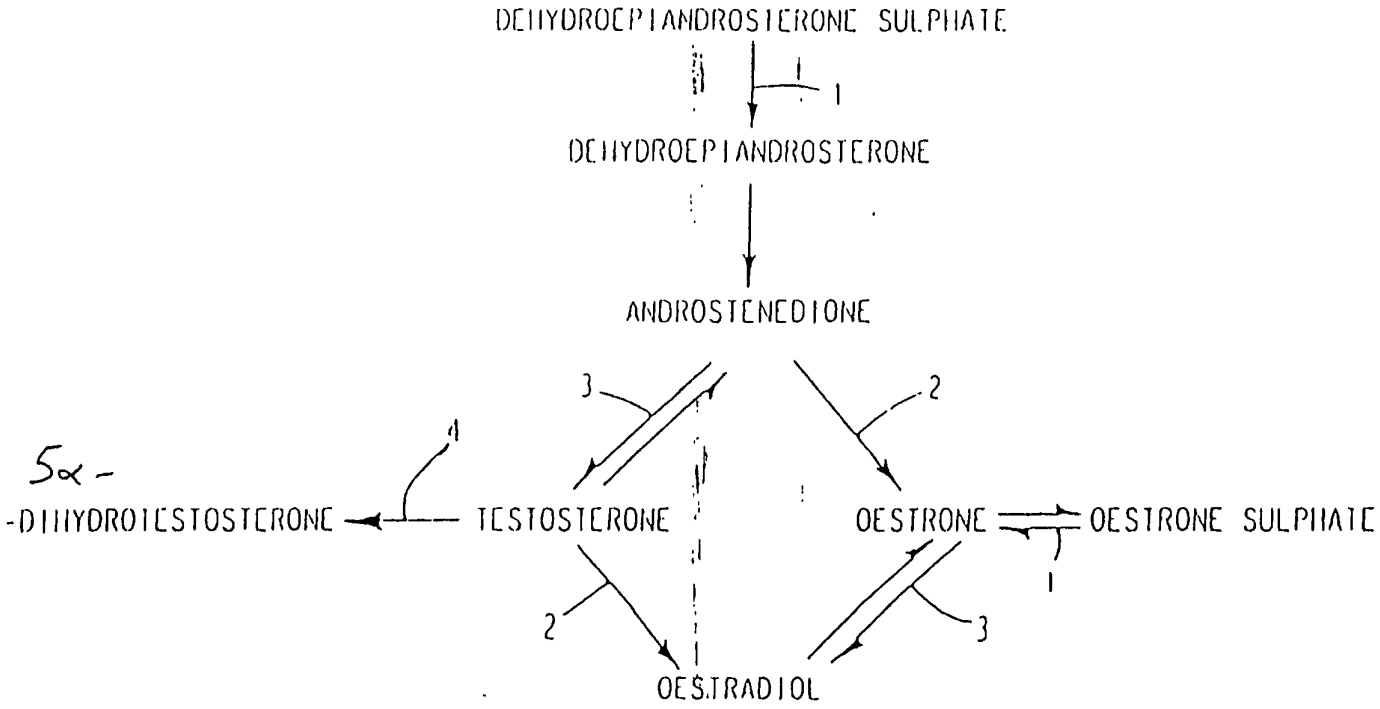
ABSTRACT

A COMPOUND

A sulphamate compound is described. The compound is suitable for use as an inhibitor of both oestrone sulphatase activity and aromatase activity. A preferred compound has the general formula II wherein F represents a phenolic ring structure (the first ring structure), J represents the third ring structure, I represents a phenolic ring structure (the second ring structure), G is an optional double bond, H is a link joining the second ring structure to the third ring structure, and Y represents a suitable second group; wherein any one of ring structures F, J and I has bound thereto a sulphamate group.

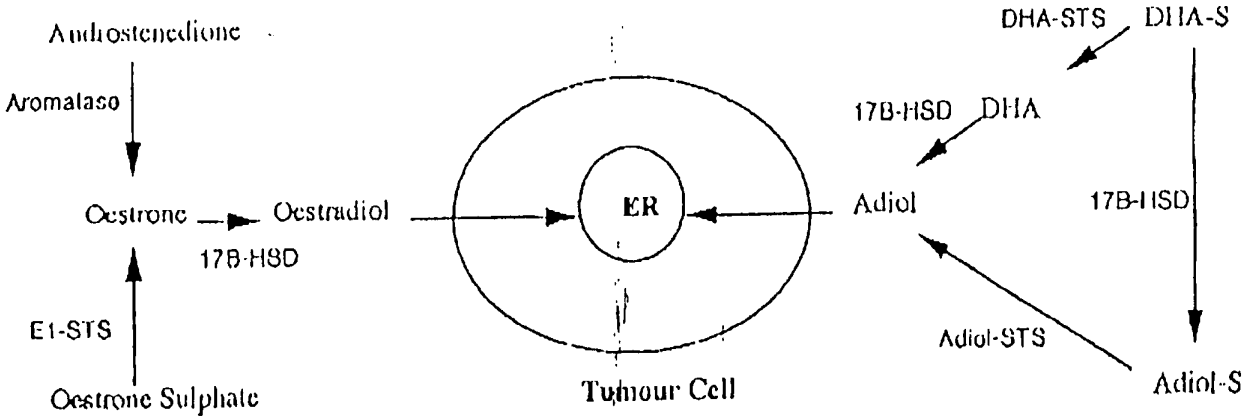
Fig 3b



KEY ENZYMES IN STEROIDOGENESIS:-

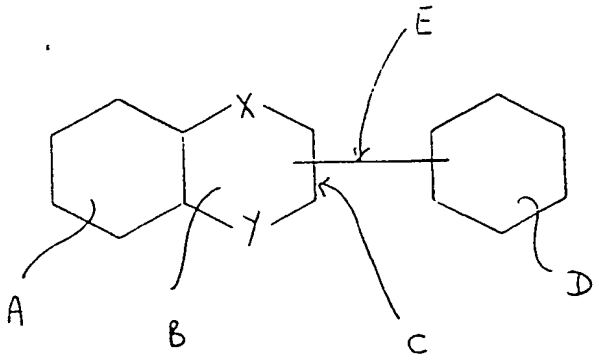
1. SULPHATASE 2. AROMATASE 3. DEHYDROGENASE 4. 5α REDUCTASE

Origin of Oestrogenic Steroids in Postmenopausal Women



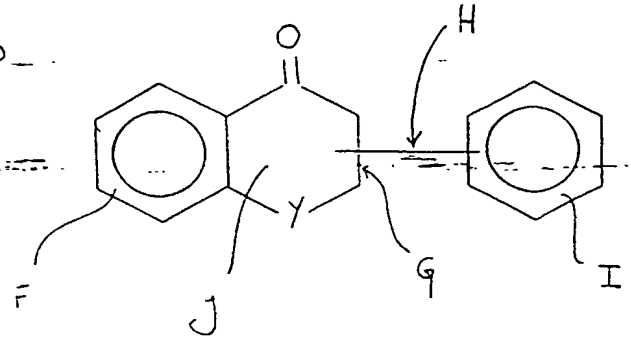
ER = Oestrogen Receptor, DHA / -S = Dehydroepiandrosterone / -Sulphate, Adiol = Androstenediol, E1-STS = Oestrone Sulphatase , DHA -STS = DHA-sulphatase, Adiol-STS = Adiol Sulphatase, 17B-HSD = Oestradiol 17B-hydroxysteroid dehydrogenase

Fig 3a



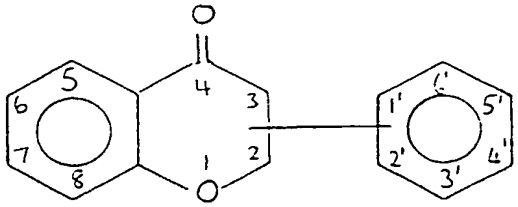
I

Fig 3b



II

Fig 3c



III

Fig 4

IV

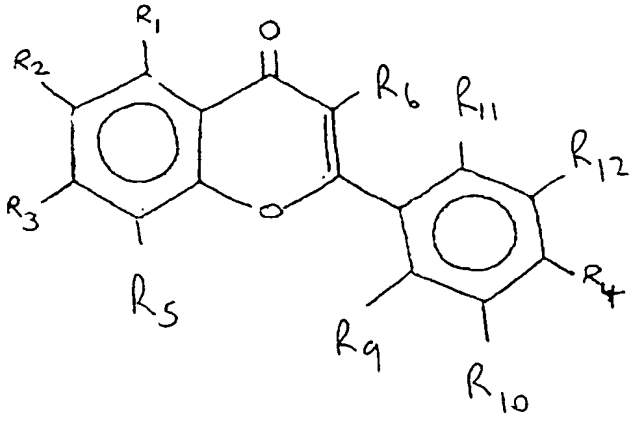


Fig 5

V

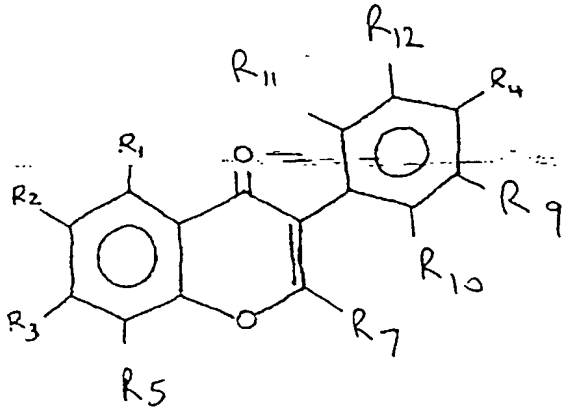
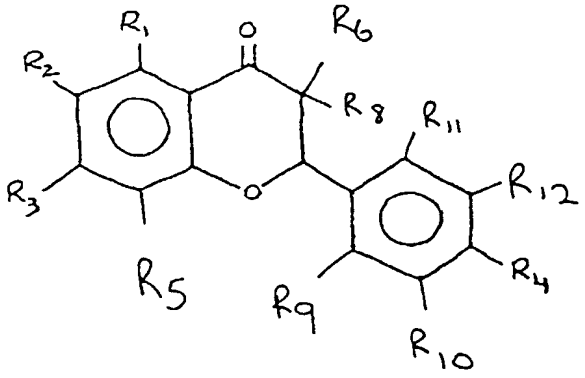


Fig 6

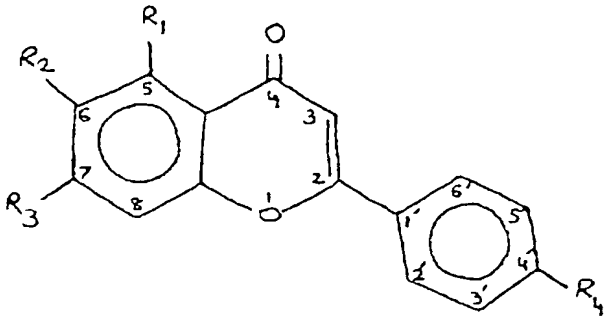
VI



FLAVONES

Fig 7

VII

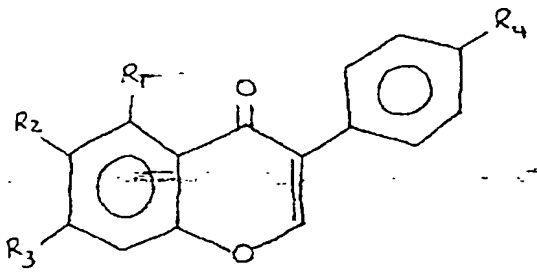


	R_1	R_2	R_3	R_4
1	H	OH	H	H
2	H	OSO_2NH_2	H	H
3	H	H	OH	H
4	H	H	OSO_2NH_2	H
5	OH	H	OH	H
6	OH	H	OSO_2NH_2	H

ISOFLAVONES

Fig 8

VIII

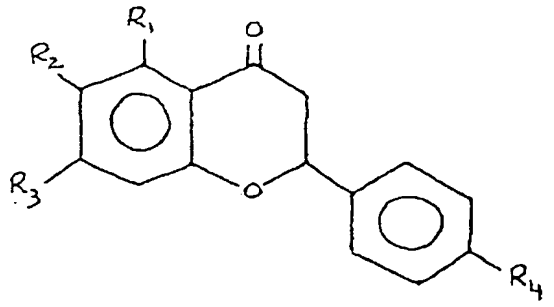


	R_1	R_2	R_3	R_4
9	OH	H	OH	OCH_3
10	OH	H	OSO_2NH_2	OCH_3

FLAVANONES

Fig 9

IX



	R_1	R_2	R_3	R_4
7	OH	H	OH	OH
8	OH	H	OH	OSO_2NH_2

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③ L Young & Co